Local and Regional Applications of Hydrogen Peroxide in the Control of Clostridial Myositis in Rabbits


Department of Microbiology, Graduate Division at the College of Dentistry and Charles A. Sammons Research Division, Baylor University Medical Center, Dallas, Texas

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The intra-arterial infusion of hydrogen peroxide has been used as a method for producing a hyperoxic environment in experimental animals for the treatment of experimentally induced clostridial myositis. Eighty-five rabbits were employed in this study; 43 were controls and 42 were experimental animals. In the experimental study, 21 animals were treated with hydrogen peroxide by each route of administration. In this group, 52.4% of the animals receiving the intra-arterial infusion and 66.6% receiving intramuscular clysis survived. There were no survivors past 72 hr in the control group.

Because of the nature of Clostridium perfringens and the disease it produces, antibiotics and antisera are effective therapy when the infection is still local; the advanced or systemic disease, however, must be managed in a more aggressive manner. The progress of the disease is predicated on ischemia and subsequent anoxia which allows the anaerobic organism to proliferate and produce its toxin; this in turn spreads the area of necrosis and anoxia so that the disease, once started rapidly, reaches a state at which it becomes self-perpetuating and fulminating. Since the organisms involved are anaerobes, the most direct approach to the problem would be to increase the oxygen tension in the area to a point which is not conducive to the survival of the organisms. This can be accomplished conveniently by three methods: (i) breathing oxygen at increased pressures (4, 5, 9, 12, 13); (ii) the administration of hydrogen peroxide intra-arterially (1–3, 6, 7, 10, 11); or (iii) the intramuscular (im) administration of hydrogen peroxide in a physiological solution delivered directly to the invaded area.

In earlier studies reported from this laboratory, it has been demonstrated that biological fluids containing catalase and peroxidases become hyperoxygenated after their degradation of exogenous hydrogen peroxide. It has been shown in vivo that the breakdown of hydrogen peroxide progresses at a sufficiently rapid rate to prevent any unacceptable side reactions, and that oxygen supplied in this fashion is in a form which is metabolically usable by the animal. With these data in mind, the present study was designed to determine the efficacy of oxygen drenching by either intra-arterial infusion or im administration of hydrogen peroxide in the control or cure of clostridial myositis in rabbits.

MATERIALS AND METHODS

Animals. Female, albino, Giant New Zealand rabbits weighing between 2 and 2.7 kg were used throughout the study.

Isolation and identification of Clostridium perfringens. The microorganism used to produce the infection was Clostridium perfringens, type A, isolated from a patient in Baylor University Medical Center with an active case of gas gangrene. The primary isolate was made in thioglycolate broth. The identification was made by characteristic growth on blood agar, sugar fermentations, and stormy fermentation in milk. The cultures were periodically checked for purity by Gram stain and characteristic growth on blood agar plates. This microorganism was maintained in stock culture under anaerobic conditions with passage every 48 hr into Brain Heart Infusion (Difco).

Anaerobic culture procedure. An anaerobic incubator from the National Appliance Co., Portland, Ore., was used throughout this study. All cultures were degassed for a minimum of 1 hr under 29 inches (73.7 cm) of mercury vacuum and flushed three times with nitrogen to assure very low oxygen tension in the media into which the cultures were to be inoculated. The tubes were then subjected to 24 inches (60.9 cm) of mercury vacuum, and pressure was allowed to equilibrate to atmospheric pressure with nitrogen gas. A nitrogen flow of 4 liters per hr was maintained through the chamber during incubation at 37.5 C.
Production of disease in animals. To produce the disease in animals, 0.5 ml of a 24-hr culture and 0.5 ml of a 72-hr culture were mixed with 0.5 ml of 1:1000 epinephrine and injected im into the depilated, left hind limb of the rabbit. This represented approximately 5 million bacteria. The epinephrine when given im acts as a severe vasoconstrictor and was included to produce a local area of ischemia and anoxia in which the microorganisms might proliferate (8). The host animal developed a lesion which became crepitus and edematous within 4 to 8 hr, and the animal was sacrificed at 12 hr. The hind limb was washed repeatedly with Betadine (Physicians Products Co., Inc., Petersburg, Va.), draped, and prepared for aseptic necropsy. A 5-cm incision was made in the hind limb and the muscles were dissected out; sections of the infected area showing gaseous formation and necrosis were removed and each section was placed in 100 ml of Brain Heart Infusion. These cultures were incubated for 12 hr at 37.5°C under anaerobic conditions. At the end of this time, the tissue was removed by filtration through four layers of sterile gauze, the cultures centrifuged, and the bacteria collected and re-suspended in a portion of the original culture media. This material was stored under nitrogen for use in the production of experimental gas gangrene in rabbits. The purity of the culture was established after each such passage by cultures on blood-agar plates to observe for colony type, double-zone hemolysis, and Gram-stain examination. This procedure for obtaining virulent organisms was completed for each experimental animal procedure reported herein. The organisms were quantitated by direct count through use of the red blood cell diluting pipette and hemocytometer, and by microhematocrit.

Titration of L. volatit at 72 hr for Baylor University Medical Center strain of C. perfringens. Groups of five animals each were given graded numbers of organisms with epinephrine as previously described. The first group was given 2.5 x 10^5 organisms; the second group, 3.5 x 10^6 organisms; and the third group, 5 x 10^6 organisms. All animals were observed until their expiration or for 7 days.

Experimental protocol. A total of 85 animals divided into five groups was used: Group I, untreated controls (25 animals); Group II, intra-arterial infusion controls (10 animals); Group III, im clysis controls (10 animals); Group IV, those with intra-arterial treatment with hydrogen peroxide (21 animals); and Group V, those with im clysis of hydrogen peroxide (21 animals).

Intra-arterial control and experimental animals. In this series, all animals were anesthetized with pentobarbital sodium (40 mg/kg), given intravenously into the marginal ear vein. The animals were shaved on the medial aspect of the right hind leg and on the lateral aspect of the left hind leg. The right limb was then washed repeatedly with Betadine; the animal was draped and prepared for aseptic surgery. A 2-cm incision was made in the right thigh, and the femoral artery was isolated. A PE-10 catheter was introduced into the artery and passed retrograde to a level of 2 cm above the bifurcation of the aorta into the iliacs and was secured in place with a purse-string suture. Two stay sutures were placed around the catheter at a point distal from its insertion. One was fixed to the adventitia and the second to the muscle fascia. The limb was closed in two layers with 5-0 silk for muscle closure and 3-0 cotton for skin. The catheter was flushed with heparin and flame-sealed. At the completion of surgery on all 10 animals, they were inoculated with the organisms as described and divided arbitrarily into two groups of five each, five control and five experimental animals.

Control and hydrogen peroxide-treated animals with im clysis. The animals in this series of studies were anesthetized with pentobarbital sodium (40 mg/kg) by the marginal ear vein, and the medial aspect of the left hind limb was clipped and depilated. The area was washed repeatedly with Betadine, draped, and prepared for catheter implantation. In the previous groups, 10 animals were treated in this group per series. A PE-50 catheter was inserted through a trochar which had been introduced into the muscle starting from the ischiotuberosity and running to the midpoint of the hind limb. The trochar was withdrawn and the catheter sutured in place with 3-0 cotton. The animals were then inoculated with the organisms as described above and divided into two groups of five animals each, experimental and controls.

All animals in all series had the left hind limb depilated and were allowed food and water ad libitum during the experiment. All animals were housed in restraining cages which allowed no more than a few inches of movement in any direction. These cages were equipped with wire mesh tops through which the catheter was passed from the animals to the outside of the cage. This was done so that the catheters could be maintained in place and intact during the time of study.

Treatment of experimental and control animal. All animals in Groups II through V were treated with the following schedule. The first treatment was given at 2 hr after inoculation, then the treatments occurred every 4 hr for a total time lapse of 28 hr, which included seven treatments. The intra-arterial control and experimental animals were treated in the following manner. The intra-arterial control animals were infused through the PE-10 catheter with 10 ml of “Ionomol-T” (Abbott Laboratories, North Chicago, Ill.) which contained 0.25 mg of Priscoline (Ciba Pharmaceutical Co., Summit, N.J.) delivered at a rate of 0.25 ml/min. The animals treated intra-arterially with hydrogen peroxide were given 10 ml of 0.24% hydrogen peroxide which contained 0.25 mg of Priscoline delivered at 0.25 ml/min. The im clysis control and hydrogen peroxide-treated animals were given 10 ml of “Ionomol-T” or 0.12% hydrogen peroxide at a rate of 0.5 ml/min.

Results

The determination of the LD₅₀ₐₙ₁, 72-hr titration with C. perfringens is in Table I. After the LD₅₀ₐₙ₁ at 72 hr had been determined, 13 additional untreated controls were infected with this dose during the study to insure consistent results.

The experimental animals were divided into
four groups, i.e., intra-arterial experimental (H₂O₂) animals, their controls, intramuscular experimental animals, and their controls. These studies were completed in four sets of paired experiments consisting of five animals in each group of tests and controls. The remainder of the study, including 22 animals in each of the two experimental groups (H₂O₂ animals), was completed in two additional series (Table 2). There were no survivors past the 72-hr period in any of the control groups, whereas 52.4 and 66.6% of the animals survived in the intra-arterial and intramuscular hydrogen peroxide groups, respectively. In addition, the majority of the animals expiring in the experimental group were lost within the first 24 hr, whereas the greatest animal loss in the control groups occurred between 24 and 48 hr.

All animals in the control group developed the typical gas gangrene disease as shown in Fig. 1 and 2. Figure 1 is a photograph of an animal taken 18 hr after the im introduction of the microorganisms with epinephrin. Crepitation and edema were evident at the injection site within 2 hr and steadily progressed with time. The edema, the focal area of hemorrhagic necrosis, and the spread of gas from the leg up along the abdominal wall can be seen. At 24 hr, the area of hemorrhagic necrosis extended with the classical skin discoloration and with the spread of the crepitus lesion involving the entire hind limb. Upon opening this lesion, an area of gelatinous necrosis involving the subcutaneous tissue and liquid hemorrhagic necrosis of the underlying muscle was found. This lesion is characterized by numerous gas bubbles and lipid globules in the fluid (Fig. 2).

In the animals receiving hydrogen peroxide by either route, the lesions were a focal area of necrosis which developed with time into a well-defined abscess. The border between the healthy tissue and the abscess was clearly evident. Figures 3 and 4 are photographs which compare the control and experimental intramuscular animals. Figure 3 is a view of the control clysis animal showing the typical skin discoloration, the gas infiltrate, and the area of hemorrhagic necrosis and edema. Figure 4 shows an experimental animal treated at the same time as the control in Fig. 3. At this time, there was very little gross change except for some edema.

A photograph of the intra-arterial control (Fig. 5) shows the massive area of hemorrhagic necrosis, edema, and gas formation which spread from the hind limb and extended along the abdominal wall. The animal in Fig. 6 was intra-arterially treated with hydrogen peroxide at the same time as the rabbit in Fig. 5; it had a focal area of edema and possible necrosis with no other gross changes at that time.

It became evident during the study that the rate at which the spread of the disease was halted and at which the disease subsequently healed was more rapid than expected in the hydrogen peroxide-treated animals. Tissue sections were studied from various phases of the disease, and photomicrographs of these sections were made (Fig. 7 and 8). Figure 7 was made from a hematoxylin and eosin stain of a section of muscle taken 8 hr after the injection of the Clostridium perfringens strain. The degree of destruction by gas formation and the general disturbance in the normal muscle architecture is obvious from this picture. A colony of C. perfringens can be seen adjacent to several large gas bubbles.

Figure 8 is a photomicrograph of tissue taken

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**Table 1. Titration of i.Δ00 at 72 hr in rabbits for Clostridium perfringens**

<table>
<thead>
<tr>
<th>No. of microorganisms injected (millions)</th>
<th>No. of animals</th>
<th>No. of animals expired by 72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3.5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>5.0</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* Baylor University Medical Center strain.

**Table 2. Survival time for rabbits after intramuscular injection of 5 × 10⁴ Clostridium perfringens and 0.5 ml of 1:1,000 epinephrin**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Animals surviving</th>
<th>Percentage survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (untreated controls)</td>
<td>23</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Group II (intra-arterial controls)</td>
<td>10</td>
<td>8</td>
<td>0.0</td>
</tr>
<tr>
<td>Group III (intramuscular clysis controls)</td>
<td>10</td>
<td>8</td>
<td>0.0</td>
</tr>
<tr>
<td>Group IV (intra-arterial hydrogen peroxide)</td>
<td>21</td>
<td>14</td>
<td>52.4</td>
</tr>
<tr>
<td>Group V (intramuscular clysis hydrogen peroxide)</td>
<td>21</td>
<td>16</td>
<td>66.6</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td></td>
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</tbody>
</table>
FIG. 1. Photograph taken 18 hr after the im injection of 5 million microorganisms and 0.5 ml of epinephrine. Note the gas invasion extending from the hind limb along the abdominal wall and the focal area of hemorrhagic necrosis at the injection site.

FIG. 2. Photograph of a surgically opened lesion taken 24 hr after the im injection of 5 million microorganisms and 0.5 ml of epinephrine showing gas bubbles and lipid globules so frequently observed in the control series.
FIG. 3. Close-up view of the control instillation animal showing areas of skin discoloration, edema, gas formation, and hemorrhagic necrosis.

FIG. 4. Photograph of an animal receiving instillation of hydrogen peroxide taken 36 hr after the introduction of Clostridium perfringens showing very little evidence of disease at this time, with the exception of some edema.
FIG. 5. Close-up view of the control intra-arterial infusion animal taken 36 hr after the im injection of Clostridium perfringens. Note the large area of gas, edema, and hemorrhagic necrosis.

FIG. 6. Photograph of an animal treated by intra-arterial infusion of hydrogen peroxide taken 36 hr after the im injection of the microorganisms in epinephrine.
Fig. 7. Photomicrograph of a section of muscle taken 8 hr after the im injection of 5 million Clostridium perfringens and 0.5 ml of epinephrine. Note the large gas bubbles, the colony of microorganisms, and the extent of muscle damage.

Fig. 8. Photomicrograph of muscle taken 4 days after the introduction of the microorganisms from an animal which had been treated by the im cystis of hydrogen peroxide. Note the discrete area described by the fascial plane showing areas of granulation and early fibrosis on one side of the plane and relatively normal muscle on the other.
4 days after the infection from an animal treated with im clysis of hydrogen peroxide. In this figure, normal muscle is adjacent to an area of granulation and necrosis; the two are separated in one area by a fascial plane. It is of interest to note that the disease apparently did not cross this fascial barrier.

Sections prepared from animals treated by the intra-arterial route were similar, if not identical, to those described for the im clysis series.

Acknowledgments

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Literature Cited


